Plant Physiol. (1989) 91, 800–803
0032-0889/89/91/0800/04/$01.00/0

Received for publication November 22, 1988
and in revised form June 8, 1989

Communication

Reflected Far-Red Light Effects on Chlorophyll and Light-Harvesting Chlorophyll Protein (LHC-II) Contents under Field Conditions

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ABSTRACT

The influence of various colors of soil cover (mulch) on the far-red/red (FR/R) ratio in upwardly reflected light and on concentrations of chlorophyll (Chl) and light-harvesting Chl protein (LHC-II) were measured under field conditions. The FR/R ratios above green surfaces were higher than over white surfaces. Even though plants (*Gossypium hirsutum* L. cv PD-1) were grown in full sunlight, those that received higher FR/R ratios in upwardly reflected light were taller and had thinner leaves with higher concentrations of Chl and LHC-II. A controlled environment experiment showed FR/R control of Chl and LHC-II concentrations. The results illustrate the importance of spectral distribution of reflected light on plant growth and a potential means of altering the chemistry of leaf crops under field conditions.

Controlled environment studies have shown that the ratio of FR[1] relative to R light acts through the phytochrome system to regulate stem elongation (6); leaf shape, thickness, and Chl concentration (16); and chloroplast structure including the number and size of grana and the Chl *a/b* ratio (14). The FR/R ratio received during leaf development has been associated with photosynthetic efficiency of the leaves (16), and phytochrome involvement in the adaptation of the photosynthetic mechanism (12, 14). Other studies show that genes coding for LHC-II (4, 7, 20) and NADPH-dependent protochlorophyllide oxidoreductase (2, 19) are regulated by phytochrome. LHC-II and Chl follow the same patterns of accumulation in greening seedlings (3, 8, 17, 18).

Field studies have shown that plants are able to sense and adapt to competition from other plants by measuring and responding to the amount of FR reflected from leaves of the competing plants (11). Leaves absorb most of the incoming visible light and transmit or reflect nearly all of the FR, causing plants in higher-density populations to receive a higher FR/R ratio, which acts through the phytochrome system to regulate plant developmental processes to favor survival among the perceived competition (11, 12). For example, plants in higher-density populations receive higher FR/R ratios and develop longer stems, a characteristic that increases the probability of keeping some leaves in sunlight.

Since plants respond to the FR/R ratio and they cannot discern the source of an altered ratio, variously colored soils and soil surface covers (mulches) have been studied to determine effects on spectral distribution of upwardly reflected light and the associated effects on plant development (15). The spectral distribution of upwardly reflected light (particularly the FR/R ratio) can influence photosynthate partitioning, which alters shoot/root ratios and the amount of nodulation of soybean *(Glycine max* L.) (10) as well as the fruit yield of tomatoes *(Lycopersicon esculentum* Mill.) (5). The present research was undertaken to study the influence of the FR/R ratio in upwardly reflected light on Chl and LHC-II content under field conditions.

MATERIALS AND METHODS

Field

Plant Material and Growth Conditions

Cotton *(Gossypium hirsutum* L. cv PD-1) plants were grown on irrigated plots of Norfolk loamy sand (Typic Paleudults) at the Coastal Plains Research Center near Florence, SC. Plots were covered with 6-m x 30-m sheets of black plastic. Each sheet was divided into 4.5-m long subplots that were painted with various colors of exterior enamel to provide the desired reflected light spectra. Four parallel rows (1 m apart) were marked and 5-cm diameter holes were cut in the plastic at within-row distances of 0.3 m. There were three replicate plots, each with five subplot colors. Four seeds were sown in each hole on May 9, 1988. When the seedlings were in the cotyledon stage, all but one per hole were removed by cutting below the cotyledons. In this manner, roots of the remaining plants were not damaged, and the seedlings were exposed to the various patterns of reflected light from the time of emergence. Leaf samples were collected at approximately 1000 h on June 21, 1988. The uppermost fully expanded leaf was taken from two randomly selected plants from each of the three replicate subplots for each of the five soil surface colors.

[1] Abbreviations: FR, far-red light; LHC-II, the major light-harvesting Chl *a/b* binding protein complex of PSII; R, red light; DMF, N,N-dimethyl formamide; SRID, single radial immunodiffusion.

Leaf samples were immediately placed in ice, and they were transferred to the laboratory in approximately 15 min. Means for Chl, Chl *a/b*, and LHC-II are based on six replicate determinations for each soil surface color treatment.

Plant Morphology

Stem lengths are means for 20 plants from each of three replicates per color treatment. Fresh weights per area of leaf were obtained with two leaf plugs (taken with an 18 mm diameter cork borer) per sampled leaf. One plug was taken from each half of each of the leaves.

FR/R Ratios in Reflected Light

Spectral distributions of upwardly reflected light were measured 10 cm above the variously colored surfaces to determine effects of mulch color on FR/R ratio in the seedling establishment zone. A LiCor[2] Spectroradiometer LI-1800 equipped with a remote light collector on a 1.5-m fiber optic probe was used to make measurements at 5-nm intervals between 400 and 850 nm. A reference spectrum was obtained by measuring direct sunlight. Spectral irradiances at 735 and 645 nm were used to calculate the FR/R ratios. These values were used because they approach the peaks for phytochrome action spectra in green plants; 645 nm was used instead of 660 nm because Chl competition for light at 660 nm (the approximate phytochrome absorbance maximum *in vitro)* shifts the action peak to about 645 nm in green plants (13). The FR/R ratios shown in this report are relative to the ratio in direct sunlight, which was arbitrarily assigned a value of 1.00. The rationale for this approach is that plants are adapted to sunlight, and a FR/R ratio that deviates from that in direct sunlight might signal the plant to activate or repress genes that regulate adaptation to the altered light environment (12).

SRID Analysis

Frozen leaf tissue was homogenized using a Brinkman polytron in 50 mM NaH_2PO_4 (pH 7.0), 2 mM PMSF in a ratio of 0.1 g of tissue per mL of buffer. SDS was then added to the extract to a final concentration of 2%. The extract was heated at 50°C for 15 min and the insoluble material was then removed by centrifugation at 7100*g* for 10 min at room temperature. No detectable protein could be released by a second extraction of the insoluble material. Extracts were stored at -80°C. Before analysis of the LHC-II content, the extracts were thawed and 20% Triton X-100 added to a final concentration of 4%. SRID analyses were performed as described previously (17) using rabbit antibodies specific for LHC-II (17). Purified LHC-II (17) from pea was used as the SRID standard. Pea LHC-II standards from 0.4 to 3.0 μg of protein were used to construct the standard curves for comparison with tissue extracts. LHC-II concentrations were determined relative to the amount of protein found in the pea standard.

[2] Mention of trademark, proprietary product, or vendor anywhere in this paper does not constitute a guarantee or warranty of the product by the Georgia Institute of Technology or the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Controlled Environment

Soybean [*Glycine max* (L.) Merr. cv Coker 338] seedlings were used because material was available from an on-going experiment, and because previous experiments with soybean (10) and cotton over variously colored mulches gave the same pattern of morphological development. The plants were started and grown in a vermiculite-potting soil (3:1, v/v) mixture; 3-L pots were used. Five seeds were sown per pot. Seeds were germinated at 28°C. After emergence, the seedlings were thinned to two per pot. All pots were watered with half-strength Hoagland nutrient solution (9) twice per week throughout the experiment. All plants received the same treatment except for R and FR at the end of each day to put phytochrome predominantly in the Pfr or the Pr form, respectively, at the beginning of the night.

All plants were grown in the same controlled-environment chamber at 25°C with 12-h d of cool-white fluorescent light at 520 μmol m^{-2} s^{-1}. There was no plastic or glass barrier between the fluorescent lamps and the plants. At the end of the daily light period, plants were exposed to either 5 min of R (3.6 W m^{-2} in the 600–700 nm waveband) or to 5 min of FR (3.6 W m^{-2} in the 700–770 nm waveband) at 25°C, then returned in darkness to the growth chamber for the remainder of the 12-h night. The R and FR radiation units were as described in earlier studies (14). Daily R and FR treatments began when seedlings reached the unifoliolate leaf stage. Leaf samples were taken after 20 consecutive days of treatment. They were measured for area on a LiCor-3100 area meter, weighed, quick frozen, freeze-dried, ground to pass a 0.5 mm mesh screen, and stored in darkness at -50°C until they were analyzed. Chl determinations were as described by Andersen *et al.* (1), and LHC-II determinations were very similar to those used for cotton (above), except that adjustments were made for freeze-dried samples. For LHC-II, freeze-dried samples required a second extraction of the insoluble material to release all of the protein. No detectable protein was released by a third extraction of the insoluble material. Data are presented as means ± standard errors for six replicate measurements.

RESULTS AND DISCUSSION

Field

Reflected Light

The percentages of reflected light at 645 nm and 735 nm (relative to incoming sunlight at the same wavelengths) differed over the various surface colors (Table I). The FR/R ratio over the white surface was the same as that of incoming sunlight. All of the other colors used in this study reflected FR/R ratios greater than occurred in incoming sunlight. Because of earlier studies with the Beltsville Spectrograph (13), as well as other investigations of plant responses to population density under field conditions (11, 12), we hypothesized that even small differences in the FR/R ratio over the variously colored mulch surfaces could have significant

impact on plant development. If true, a higher FR/R ratio (as was found over the green surfaces, Table I) should signal the plants to develop longer stems, thinner leaves, and perhaps a more efficient light harvesting system.

Effects on Plant Size

Plants were shortest when grown over white surfaces (Table I) and almost as short when grown over yellow (note that white and yellow surfaces reflected the lowest FR/R ratios). These responses are consistent with previous observations with soybean (10) and southern pea [*Vigna unguiculata* (L.) Walp.] (15). The plant height differences in the present study were due to internode length since leaf number per plant did not differ significantly (data not shown).

Leaves were thickest on plants grown over white and thinnest on plants grown over green surfaces, as shown by weight per leaf area (Table I). These results are consistent with earlier controlled environment studies in which a low FR/R ratio resulted in the development of thicker leaves than were developed on plants that received a higher FR/R ratio during development (12, 16). The data support the concept that the FR/R ratio in upwardly reflected light may influence plant development in the same manner as the FR/R ratio received in different plant population densities.

Chl and LHC-II

Leaves of plants grown over white surfaces had lower Chl and LHC-II concentrations than those of plants grown over green surfaces (Table I). The Chl and LHC-II values are striking in that statistically significant ($P = 0.05$) differences existed between leaves grown over white (FR/R ratio as in sunlight) and those grown over green (with a higher FR/R ratio). The same trends in Chl and LHC-II contents were observed for bell pepper *(Capsicum annuum* L.) seedlings over colored mulches (our unpublished data). From a plant adaptation standpoint, we theorized that the photosynthetic apparatus should be very responsive to subtle variations of the light environment, such as the FR/R ratio which would be influenced in nature by competition from other plants for light.

Controlled Environment

A controlled-environment experiment was conducted to determine possible phytochrome involvement in regulation of the morphological and chemical differences that were observed in plants grown over the variously colored mulches. To control some of the variables that occur in a field, all of the seedlings were grown in the same growth chamber for 23 h and 55 min per d, followed by short exposures to R or FR at the end of the photosynthetic period. The 5 min end-of-day exposures to R and FR were given to put phytochrome predominantly in the Pfr or Pr form at the beginning of the night. In this way, we were able to study the effects of high and low FR/R ratios without altering either temperature or the light spectrum and/or fluence rate during the daily photosynthetic periods.

Plants that received FR (high FR/R ratio) at the end of each day developed thinner leaves with higher Chl and LHC-II concentrations (Table II). The FR treatment also resulted in longer internodes, as observed in an earlier study (12). These responses are consistent with growth patterns observed over green *versus* white surfaces in the field, as shown in Table I. These results (Tables I and II) support the concept that an altered FR/R ratio in upwardly reflected light over the variously colored soil surface mulches can act through the phytochrome system to regulate plant developmental processes. Nevertheless, this does not rule out contributions of other environmental variables to developmental patterns in plants growing over variously colored mulches or soil surface colors in the field.

In conclusion, our study supports the concept that altered spectral distribution of upwardly reflected light over variously colored soil covers (mulches) can influence not only the morphological development of a plant but also the Chl and LHC-II concentrations in developing leaves. It appears that the FR/R ratio in the upwardly reflected light can cause a plant to initiate characteristics that would favor survival among other competing plants, even though the plants are grown in full sunlight. The relationship between FR/R ratio received by developing leaves and the Chl and LHC-II contents of those leaves offers insight into phytochrome regula-

Table I. *Upwardly Reflected Light 10 cm above Different Colored Mulches, and Effects on Cotton Plant Size, Chl, and LHC-II*

The FR/R ratios are relative to the ratio in direct sunlight, which was arbitrarily assigned a value of 1.00.

Mulch Surface Color	Upwardly Reflected Light			Plant ht	Leaf fr wt	Chl	Chl *a/b*	Relative LHC-II Content
	R[a]	FR[b]	FR/R					
	% of sunlight		*ratio*	*cm*	*mg/cm²*	*mg/g fr wt*	*ratio*	*mg/g*
Green	7.7	10.3	1.33	53	22.5	1.70	3.40	3.12
Black	5.2	5.7	1.09	53	22.8	1.50	4.11	2.85
Red	32.8	34.2	1.04	52	23.4	1.61	3.64	2.45
Yellow	44.3	44.7	1.01	48	24.4	1.31	4.13	2.30
White	43.3	43.5	1.00	42	26.6	1.26	4.10	2.10
LSD 0.05				3	3.0	0.18	0.20	0.92

[a] 645 nm. [b] 735 nm.

Table II. *Effects of R and FR on Chl and LHC-II Contents in a Controlled Environment*

The R- and FR-treated plants were grown in the same 25°C controlled environment chamber for about 23 h and 55 min per day. Exposures to R and FR were for 5 min at the end of the 12-h photosynthetic period for 20 consecutive days. All sampled leaves developed during the 20-d treatment period.

End-of-Day (5 min)		Leaf fr wt	Chl			Relative LHC-II Content
Light	FR/R		*a*	*b*	Total	
	ratio	*mg/cm²*		*mg/g dry wt*		*mg/g*
FR	High	12.9 ± 0.1[a]	10.3 ± 0.2	2.3 ± 0.1	12.6 ± 0.3	2.48 ± 0.21
R	Low	14.4 ± 0.1	6.2 ± 0.6	1.8 ± 0.1	8.0 ± 0.6	2.08 ± 0.14

[a] Values are means ± standard errors.

tion of gene expression under field conditions. Our study is an example of the potential use of basic principles of photoregulation of plant development in a plant-soil-light management system.

ACKNOWLEDGMENTS

We gratefully acknowledge the helpful suggestions and gift of LHC-II antibody by Dr. Kent O. Burkey and encouragement of the research by Dr. P. G. Hunt. The technical assistance of Woodrow Sanders and Julie Frick with field plots and Lisa Dupree in single radial immunodiffusion analysis is also greatly appreciated.

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